Journal of Chromatography A, 1218 (2011) 6554–6560

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Enantiomer elution order reversal of fluorenylmethoxycarbonyl-isoleucine in high-performance liquid chromatography by changing the mobile phase temperature and composition

L. Chankvetadze[a], N. Ghibradze[a], M. Karchkhadze[a], L. Peng[b], T. Farkas[b], B. Chankvetadze[a,*]

[a] Institute of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, 0179 Tbilisi, Georgia
[b] Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501, USA

ARTICLE INFO

Article history:
Received 15 May 2011
Received in revised form 15 June 2011
Accepted 16 June 2011
Available online 23 June 2011

Keywords:
Enantioseparations
Chiral HPLC
Reversal of enantiomer elution order
Chiral stationary phases

ABSTRACT

In this paper the elution order reversal of enantiomers of fluorenylmethoxycarbonyl- or FMOC-isoleucine is described depending on the separation temperature and composition of the mobile phase when using the polysaccharide-based chiral column Lux Cellulose-1 in HPLC with normal-phase eluent. Reversal of the enantiomer elution order (EEO) in HPLC depending on the column temperature and content of the polar modifier in the mobile phase has been reported before in the literature. However, EEO reversal by changing the content of acidic modifier in the mobile phase seems to be described for the first time in the present work.

1. Introduction

Enantiomer elution order (EEO) is an interesting issue in chiral separations from both, practical and theoretical points of view [1–3]. In enantiomeric purity assays when the minor enantiomeric impurity has to be analyzed in the presence of the major enantiomer, it is always desirable to elute the minor component in front of the major one [4–6]. This allows more sensitive and reproducible quantification of the enantiomeric impurity [4–6]. From a theoretical point of view, understanding the fine mechanisms of EEO reversal means understanding the forces and interactions responsible for analyte-selector binding and enantioselective recognition in chiral HPLC [7].

The reversal of EEO in various chromatographic techniques has been described for last three decades and summarized in two review articles [2,3]. For instance, the EEO *a priori* reverts in any chromatographic system when a given chiral selector is alternatively used with two opposite stereochemical configurations [8]. This is not possible for natural chiral selectors available only in one stereochemical configuration. The EEO of some chiral compounds can eventually be reversed when different chiral substances (e.g., cellulose and amylose) are used as starting materials for the preparation of chiral stationary phases (CSP), with different separation modes, e.g. in polar organic and reversed-phase LC [9], when a modifier is added to the mobile phase [10], two different modifiers are used [6,11–13], or the content of a given modifier changes within a given chiral column [7,14]. In addition, several examples are described in the literature, when the EEO changes by changing the separation temperature [14–16]. Although in some previous articles conformational changes of the chiral selector under the effect of EEO reversing factors are suggested along with possible changes in the selectand/analyte interactions [7], the former is considered to be basically responsible for EEO reversal.

In the present study the EEO reversal of fluorenylmethoxycarbonyl- or FMOC-isoleucine is reported by changing the separation temperature and the content of the polar modifier in the mobile phase, but also by changing the concentration of formic acid (FA) as acidic additive in the mobile phase in a rather narrow range (0.1–0.5% (v/v)). A similar effect was observed in a parallel study performed in co-operation with Crommen and co-workers for the chiral drug amlodipine, but with a different mobile phase-CSP combination [17]. These two examples seem to be the first report on EEO reversal based on the content of acidic additive in the mobile phase.

2. Experimental

2.1. Materials

L- and D-FMOC-isoleucine (Fig. 1) and formic acid were commercially available compounds from Sigma–Aldrich (St. Louis, MI, USA). HPLC-grade n-Hexane, 2-propanol and ethanol were sup-

* Corresponding author. Tel.: +995 32 290648, fax: +995 32 913369.
E-mail address: bezhan_chankvetadze@yahoo.com (B. Chankvetadze).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.06.068

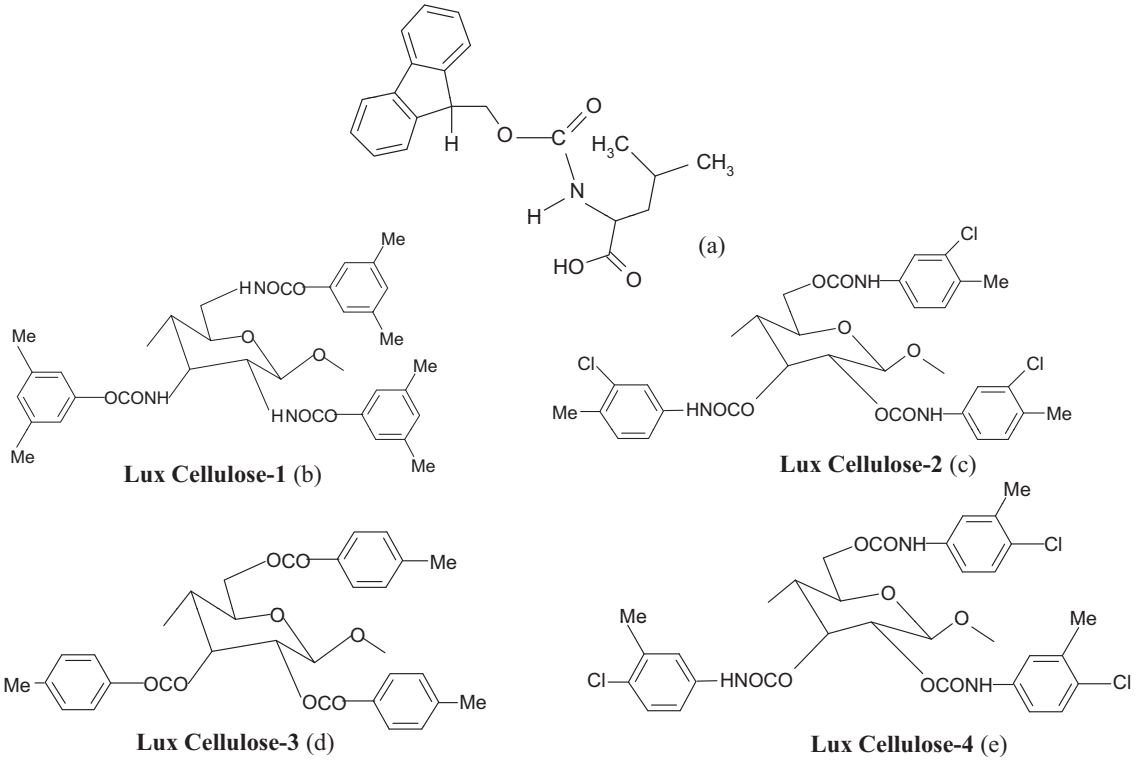

Fig. 1. Structure of FMOC-isoleucine (a) and cellulose-based chiral selectors (b–e) in the chiral columns used in this study.

plied by Karl Roth (Karlsruhe, Germany). Chiral columns used in this study (all with 4.6 mm × 250 mm dimensions and packed with 5 μm packing material) were commercially available from Phenomenex Inc. (Torrance, CA, USA) and were kindly provided by their manufacturer. The structure of the chiral selectors in Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3 and Lux Cellulose-4 columns are shown in Fig. 1.

2.2. Instruments

The chromatographic experiments were performed on an Agilent 1200 HPLC system equipped with a vacuum degasser G1354A, a binary pump SL G1312B, an autosampler G1329A, a thermostated column compartment G1316A, and a photodiode array detector G1315D from Agilent Technologies (Santa Clara, CA, USA). Chromatographic separations of a non-racemic mixture of enantiomers were performed with 1 ml/min linear flow rate of the mobile phase with various columns, eluents and at various separation temperatures as discussed below. The instrument control and data management were performed with Chemstation software.

3. Results and discussion

3.1. Effect of chiral selector and the nature of alcohol modifier on EEO of FMOC-isoleucine

In this work the effect of various separation variables including the nature of the chiral selector on EEO of FMOC-isoleucine is reported. Since the effect of multiple variables is reported deep mechanistic discussions are not provided at this stage and just references to similar effects (if any) previously reported in the literature are given. More thorough thermodynamic and mechanistic studies shall be reported in our forthcoming works on the same topic.

In the first step the EEO of FMOC-isoleucine was studied on four different cellulose-based chiral stationary phases and the reversed EEO was observed between some chiral selectors (Fig. 2). Many examples of this kind are described in the literature (see for example, Ref. [18]).

Further studies were focused on Lux Cellulose-1 column since the most interesting effects were observed on this column.

At the same concentration of 20% (v/v) of ethanol and 2-propanol as polar modifiers of the mobile phase the EEO of FMOC-isoleucine was the same on the Lux Cellulose-1 column (data not shown). However, this conclusion is accidental since the EEO of FMOC-isoleucine on this chiral column reverses depending on the concentration of 2-propanol in the mobile phase. Therefore, if compared at different concentrations of the two modifiers one could observe the opposite EEO of FMOC-isoleucine.

3.2. Effect of content of polar organic modifier on EEO of FMOC-isoleucine

After studying the effect of the nature of alcohol modifier on the EEO of FMOC-isoleucine, the content of 2-propanol was varied in the mobile phase and interesting peak coelution and the reversal of EEO was observed (Fig. 3). Similar effect was reported by Ma et al. in 2009 for a phenylalanine derivative on the amylose-based chiral column Chiralpak AD when the content of ethanol was varied in the mobile phase from 0 to 20% (v/v). The authors have explained this phenomenon based on vibrational circular dichroism (VCD) studies by some conformational change of the chiral selector [7]. In our opinion, the structural changes of the chiral analyte itself must be also evaluated. It seems interesting mentioning that Ma et al. did not observe the same effect with Chiralcel OD column which has the same chiral selector as Lux Cellulose-1, as well as with 2-propanol as polar modifier of the mobile phase [7]. Thus, the phenomena described in ref. [7] and in the present study are

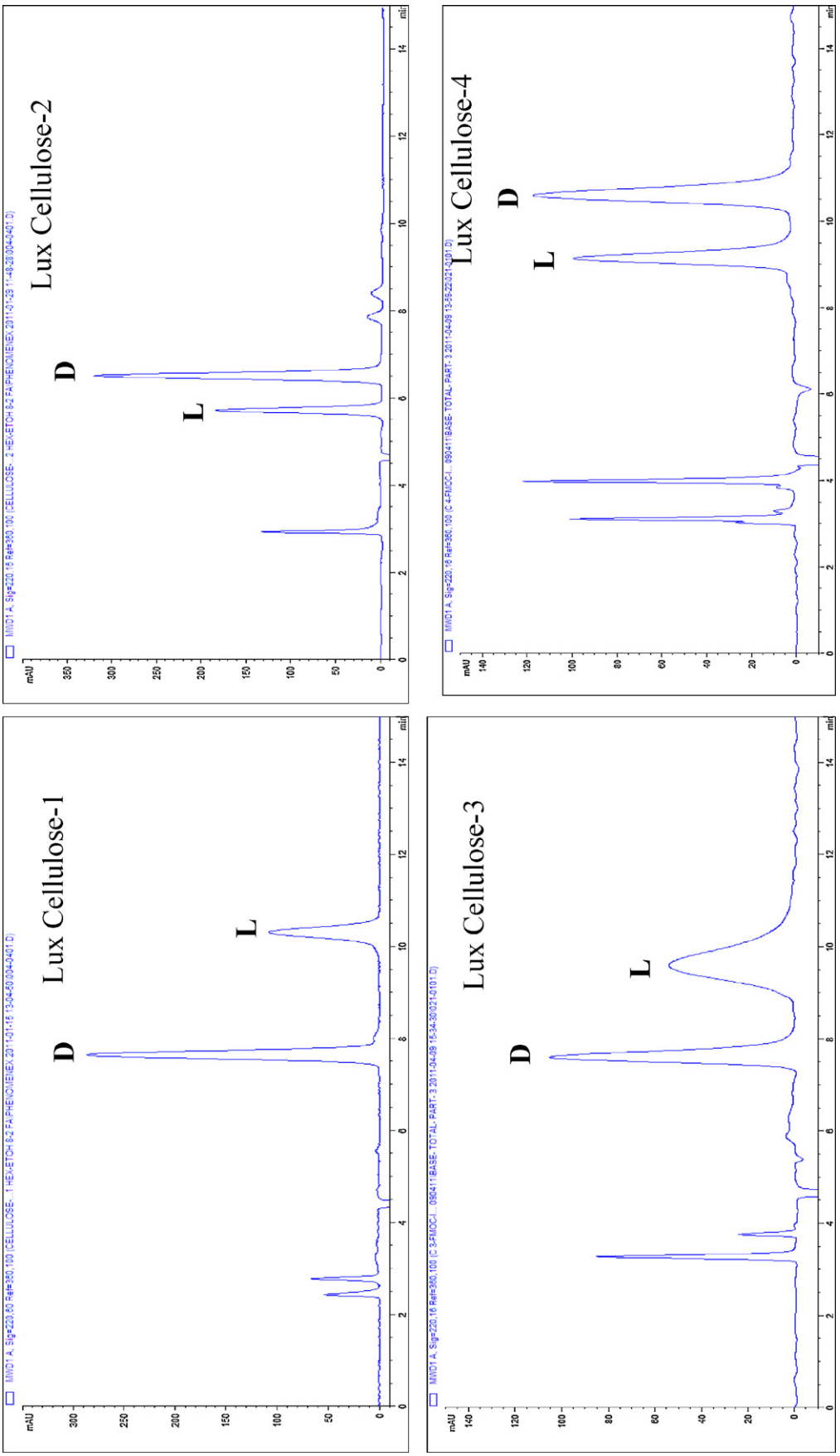

Fig. 2. The effect of the nature of chiral selector on EEO of FMOC-isoleucine. Mobile phase: n-hexane/ethanol/FA 80/20/0.1% (v/v/v); separation temperature 20 °C. For other experimental parameters see Section 2.

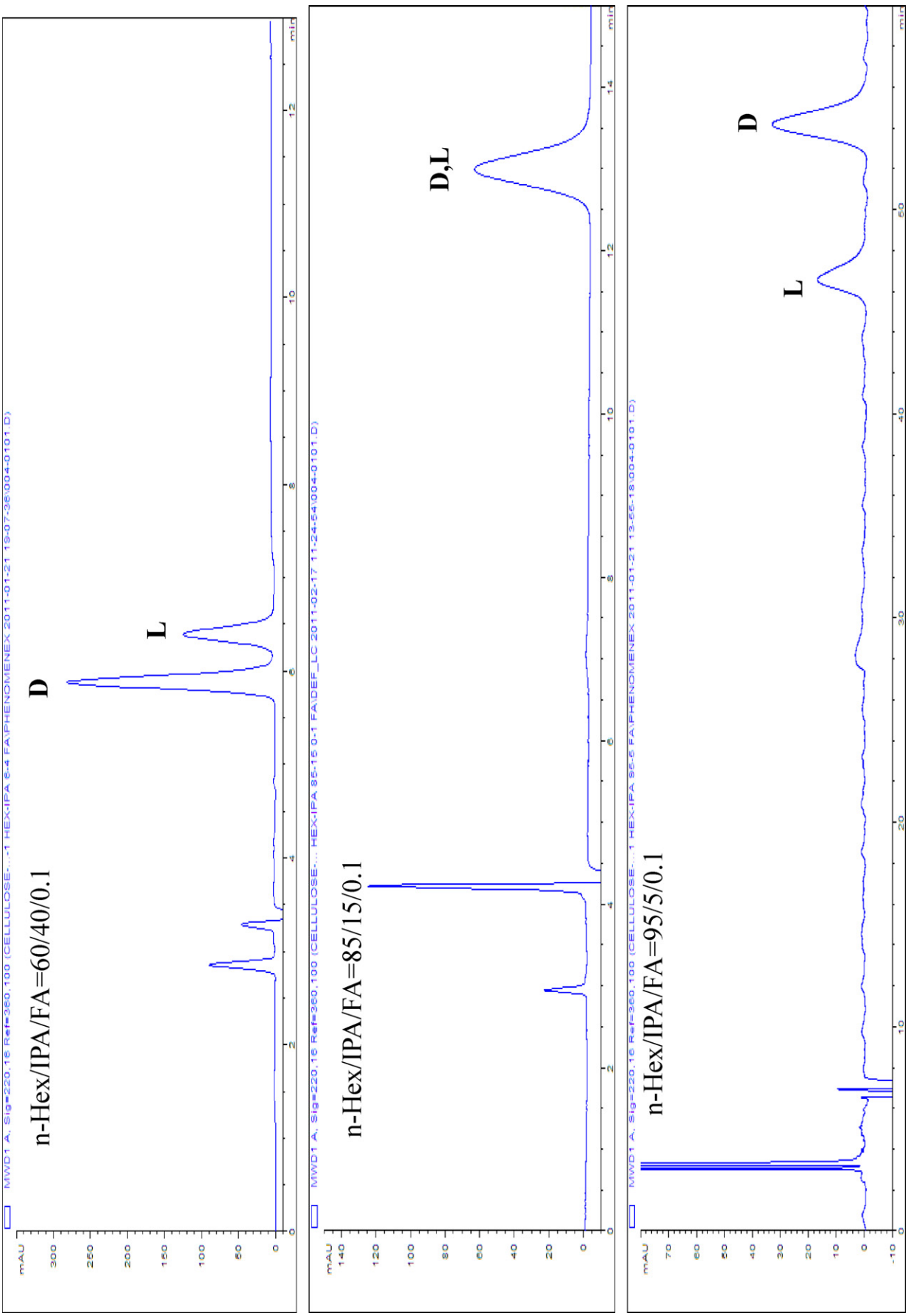

Fig. 3. Reversal of enantiomer elution order of FMOC-isoleucine on Lux Cellulose-1 by varying the content of 2-propanol in the mobile phase. The mobile phase was n-hexane/2-propanol in various ratios with 0.1% FA. Separation temperature was 20 °C. For other experimental parameters see Section 2.

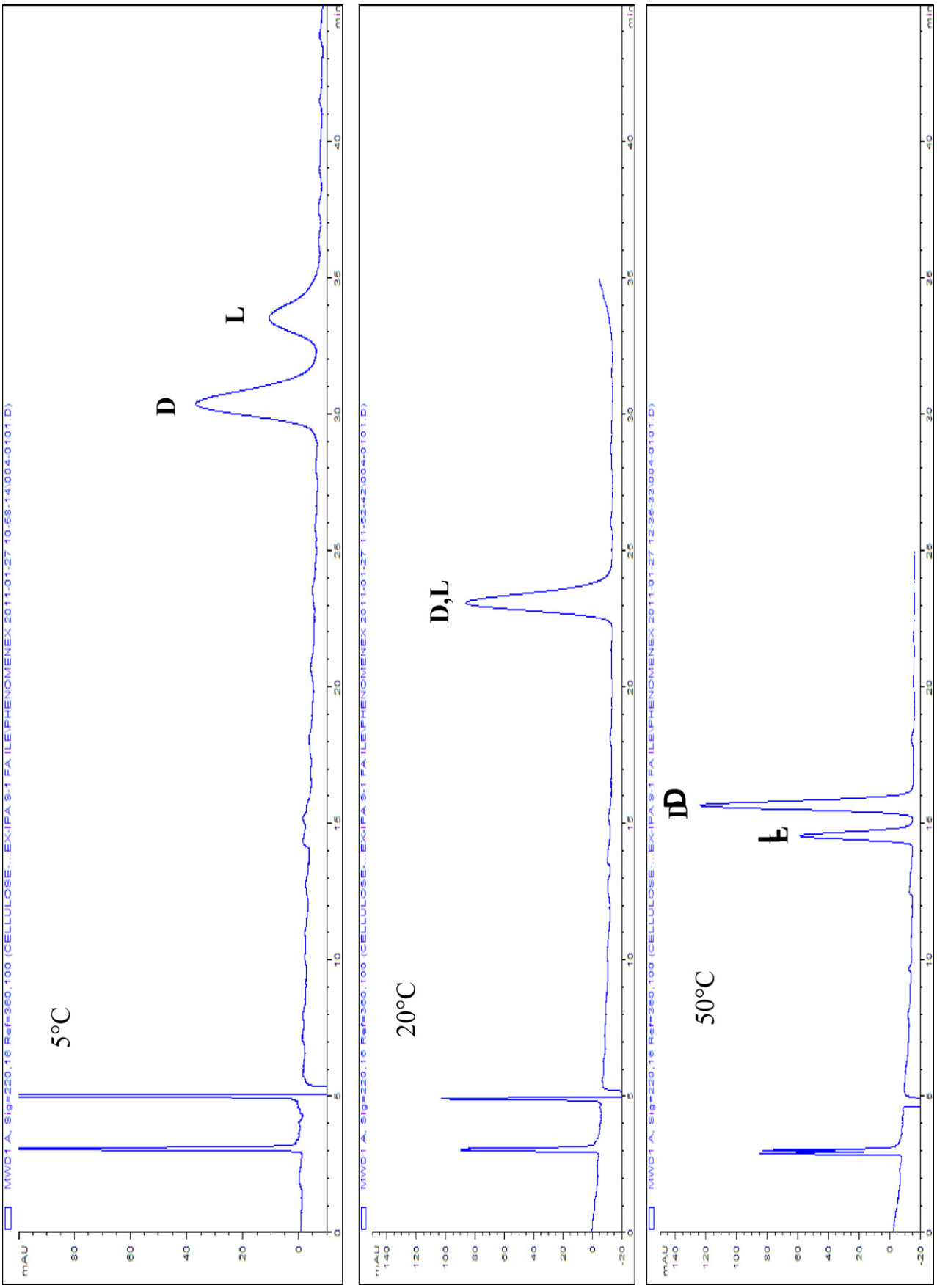

Fig. 4. Reversal of enantiomer elution order of FMOC-isoleucine on Lux Cellulose-1 by varying the separation temperature. The mobile phase was n-hexane/2-propanol/FA 90/10/0.1% (v/v/v). For other experimental parameters see Section 2.

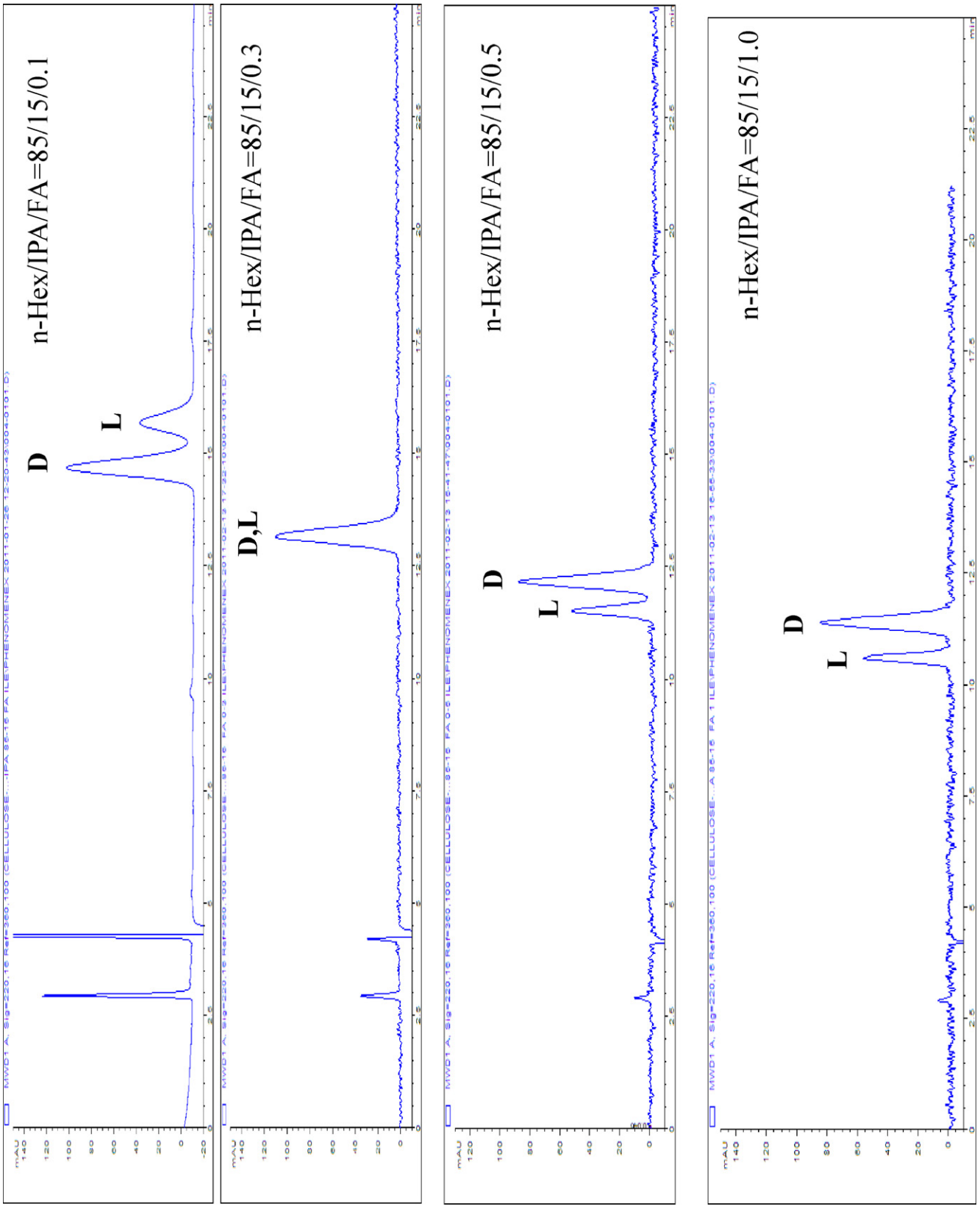

Fig. 5. Reversal of enantiomer elution order of FMOC-isoleucine on Lux Cellulose-1 by varying the formic acid content in the mobile phase. The mobile phase was n-hexane/2-propanol 90/10 (v/v) with various contents of formic acid as indicated in the figure. Separation temperature was 20 °C. For other experimental parameters see Section 2.

quite complimentary from the viewpoint of CSP and the alcohol modifier used.

3.3. Effect of separation temperature on EEO of FMOC-isoleucine

According to the well known equation (1) in chiral chromatography the enthalpy and entropy contributions to the separation process of a pair of enantiomers cancel each other out at a certain temperature and peak coelution is observed.

$$\ln \alpha = -\frac{\Delta_{R,S}\Delta H^{\circ}}{RT} + \frac{\Delta_{R,S}\Delta S^{\circ}}{R} \quad (1)$$

where $\Delta_{R,S}\Delta H^{\circ}$ and $\Delta_{R,S}\Delta S^{\circ}$ are the differences between two enantiomers in enthalpy and entropy of adsorption, respectively, onto stationary phase, R is the gas constant and T the absolute temperature.

Below this value (the so called isoenantioselective temperature, T_{iso}), the enthalpy contribution $\Delta_{R,S}\Delta H^{\circ}$ dominates the separation process while above this temperature the entropy contribution $\Delta_{R,S}\Delta S^{\circ}$ dominates. The EEO is opposite below and above the isoenantioselective temperature (T_{iso}). In most cases the isoenantioselective temperature is too high to be commonly reached, especially in HPLC. Therefore, just few examples of temperature dependent reversal of EEO are described in the HPLC literature [14–16]. After careful selection of the mobile phase conditions, the temperature-dependent reversal of EEO of FMOC-isoleucine was observed with the isoenantioselective point at the rather low temperature of 20 °C (Fig. 4).

3.4. Effect of content of formic acid additive on EEO of FMOC-isoleucine

Next, the effect of the concentration of the acidic additive formic acid in the mobile phase on the EEO of FMOC-isoleucine was studied. Although the concentration of this additive varied in a rather narrow concentration range from 0.1 to 1% (v/v), peak coalescence was observed at 0.3% FA and reversal of EEO with baseline resolution already at 0.5% of this additive (Fig. 5). With the FA concentration increased to 1% the separation factor improved further. This finding, together with our parallel observation for the chiral drug amlodipine [17], seems to be the first report on the reversal of EMO caused by changing the concentration of acidic additive in the mobile phase in a rather narrow concentration range.

4. Conclusions

This study reports the reversal of enantiomer elution order of FMOC-isoleucine based on chiral selector, content of the polar organic modifier of the mobile phase, separation temperature and for the first time, the acidic modifier content in the mobile phase. Further studies on these effects may provide valuable information for better understanding the mechanism of chiral recognition on polysaccharide-based chiral stationary phases. Furthermore, such susceptibility of enantiomer separation to the content of organic modifiers and separation temperature must be considered in chiral separation method development and optimization.

References

[1] B. Chankvetadze, Electrophoresis 23 (2002) 4022.
[2] M. Okamoto, J. Pharm. Biomed. Anal. 27 (2002) 401.
[3] B.-A. Persson, S. Andersson, J. Chromatogr. A 906 (2001) 195.
[4] J.A. Perryt, J.D. Rateike, J.T. Szczerba, J. Chromatogr. 389 (1987) 57.
[5] T.J. Wozniak, R.J. Bopp, E.C. Jensen, J. Pharm. Biomed. Anal. 9 (1991) 363.
[6] K.S.S. Dossou, P. Chiap, A.-C. Servais, M. Fillet, J. Crommen, J. Pharm. Biomed. Anal. 54 (2011) 687.
[7] S. Ma, S. Shen, H. Lee, M. Eriksson, X. Zeng, J. Xu, K. Fandrick, N. Yee, C. Sananayake, N. Grinberg, J. Chromatogr. A 1216 (2009) 3784.
[8] F. Gasparrini, D. Misiti, M. Pierrini, C. Villani, J. Chromatogr. A 724 (1996) 79.
[9] T.L. Xiao, B. Zhang, J.T. Lee, F. Hui, D.W. Armstrong, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 2673.
[10] J. Hermansson, G. Schill, in: M. Zief, L.J. Crane (Eds.), Chromatographic Chiral Separation, Marcel Dekker, New York, 1988, p. 245.
[11] J. Haginaka, J. Wakai, K. Takahashi, T. Katagi, Chromatographia 29 (1990) 587.
[12] M. Okamoto, H. Nakazawa, J. Chromatogr. 588 (1991) 177.
[13] K. Balmer, B.-A. Persson, P.-O. Lagerström, J. Chromatogr. A 660 (1994) 269.
[14] K. Balmer, P.-O. Lagerström, B.-A. Persson, G. Schill, J. Chromatogr. 592 (1992) 331.
[15] W.H. Pirkle, P.G. Murray, J. High Resolut. Chromatogr. 16 (1993) 285.
[16] A. Aranyi, I. Ilisz, Z. Pataj, I. Szatmari, F. Fülöp, A. Peter, J. Chromatogr. A, in press.
[17] K.S.S. Dossou, P.A. Edorh, P. Chiap, B. Chankvetadze, A.-C. Servais, M. Fillet, J. Crommen, J. Sep. Sci., in press, doi:10.1002/jssc.201100339.
[18] B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A 670 (1994) 39.